

Certificate of Analysis for NR-10312

Genomic DNA from Bacillus anthracis, Strain Sterne \(\Delta \text{BAA1887-2} \)

Catalog No. NR-10312

This reagent is the tangible property of the U.S. Government.

Product Description: Genomic DNA was isolated from a preparation of *Bacillus anthracis* (B. *anthracis*), strain Sterne Δ GBAA1887-2. Strain Sterne Δ GBAA1887-2 is a deletion mutant of the toxigenic acapsulate original Sterne strain (34F2), constructed by replacing codons 10 through 14 with three in-frame stop codons followed by the recognition site for *Bam*HI (to facilitate screening of the correct mutation). The remainder of the putative enterotoxin gene (GBAA1887-2) retains the wild type sequence.

Lot¹: 58253187 Manufacturing Date: 03APR2009

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1420 base pairs)	Consistent with B. cereus group	Consistent with <i>B. cereus</i> group ²
Presence or Absence of Plasmids Confirmed by PCR Amplification		
pXO1 (aat) pXO2 (at, capA, capB, capC)	Positive Negative	Positive Negative
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Content by PicoGreen® Measurement	4 to 6 μg in 25 to 100 μL per vial	5.5 μg in 39 μL per vial (142 μg/mL)
Functional Activity by PCR Amplification 16S ribosomal RNA gene Virulence markers on plasmid pXO1 (aat)	~ 1500 bp amplicon ~ 125 bp amplicon	~ 1500 bp amplicon ~ 125 bp amplicon
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 1.9	1.79
Bacterial Inactivation 10% of total yield plated on Tryptic Soy Agar with 5% sheep blood ^{3,4}	No viable bacteria detected	No viable bacteria detected

NR-10312 was produced by Tryptic Soy Broth culture of NR-10008 (Lot: 58441529). After incubation for 24 hours at 37°C and aerobic atmosphere, genomic DNA was extracted using proprietary technology.

Date: 02 SEP 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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NR-10312 58253187 02SEP2009

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ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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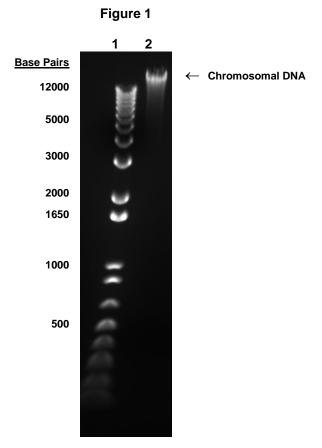
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²Bacillus cereus group species (B. cereus, B. thuringiensis, B. mycoides, and B. anthracis) cannot be classified based on 16S sequence [Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093].

³7 days at 37°C in an aerobic atmosphere

⁴An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.

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Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder

Lane 2: 200 ng of NR-10312

800-359-7370

NR-10312_58253187_02SEP2009